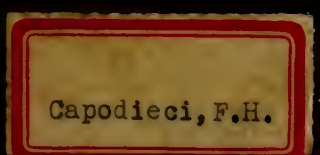

BOSTON UNIVERSITY

GRADUATE SCHOOL

Thesis

THE ROLE OF THE THYMUS IN GROWTH AND DEVELOPMENT

by

Florindo Horace Capodieci

(S.B., Boston University, 1937)

submitted in partial fulfilment of the

requirements for the degree of

Master of Arts

1938

Table of Contents

Introduction

Until the last decade it was impossible to make a definitely accurate statement concerning the function of the thymus gland. Many of the experiments performed furnished evidence for endocrine function, but this evidence was flanked by so many confusing and contradictory statements that it was not even possible to state definitely that the thymus was an endocrine gland. Rowntree's work of the past few years, while lacking confirmation, seems to give evidence that the thymus is possessed of endocrine function.

The earlier workers on the thymus gland made much of the fact that it is a large organ in the very young and is usually very small in the adult. From this they concluded that the thymus was related to growth and of little or no use in later life. For the most part, earlier work on the thymus confined itself to attempts to remove the gland with the hope of producing abnormalities in growth and development, especially of the skeleton. The administration of thymus by feeding, implantation or injection did not come until a much later date.

The work of earlier writers was instrumental in altering scientific opinion concerning the function of the thymus in spite of the fact that their experiments were often poorly controlled, performed and interpreted. For example, Klose (1910) in reviewing Friedelbein's experiment, states that one of Friedelbein's thymectomized dogs died of cachexia, which

Klose describes as a definite symptom of thymectomy. He disregards Friedelbein's statement that the animal died of an intestinal infection.

However, it seems advisable to include the work of several of these pioneers in the form of an historical review because their methods of thymus extirpation have been proven basically sound and practical by recent investigators. As a matter of fact, Friedelbein in 1858 was removing the thymus by traction throughan incision in the soft parts of the base of the neck. Rowntree (1934) recognized as the leading investigator in this field, uses Friedelbein's method with but slight variation.

The remainder of this review will be devoted to a consideration of more recent studies on the thymus gland with a somewhat detailed account of the work of Rowntree and his co-workers.

Development of the Thymus Gland in Higher Mammals

The thymus takes its origin from the ventral diverticula in higher vertebrates and from the dorsal diverticula in lower vertebrates, so it is not the same organ in the entire vertebrate series when condidered from the standpoint of its development. Hammar(1905) divides the higher mammals into three groups according to the position of the thymus. In the first group the thymus lies chiefly or entirely in the thorax. In the second group it lies in both the thorax and neck. In the third group it lies in the neck alone. The thymus of most higher vertebrates is purely entodermal in

origin being derived from the third pharyngeal pouch alone or from the third and fourth pouches. An exception to this rule is found in the pig where the thymus is said to arise not only from the third pharyngeal pouch but also from the ductus precervicalis medialis which is a derivative of the ectoderm, (Badertscher, 1914).

The thymus usually takes the form of a lobulated mass entirely surrounded by fibroelastic connective tissue. Extensions of this connective tissue divide the gland into small lobules, (Scott and Kendall, 1935). Each lobule appears to be a mass of lymphoid tissue with a darkly staining peripheral cortex of more dense lymphoid cells and a central diffuse medulla. The lymphoid cells of the cortex are regarded as lymphocytes which have migrated into this region, (JOB, 1922). Small cell masses formed of fusiform cells are found in the medulla of the lobules. These cell masses make up what are known as Hassal's corpuscles. The fact that many of the corpuscles contain calcium deposits, has led some investigators to believe that the thymus controls or affects the calcium balance of the body. This idea, as yet, has not been proven.

The thymus normally undergoes involution in the adult animal, the lymphoid tissue becoming progressively replaced by connective tissue containing fat cells.

Historical Review

Attempts to discover the function of the thymus by removal of the gland began with Restelli in 1845. He op-

erated on 72 sheep, 23 dogs and 3 calves. Everyone of his animals died within twenty days of any one of a dozen causes ranging from fever to pneumonia. No inferences regarding thymus function may be drawn from Restelli's work and it can have only historical interest. Friedelbein (1858) completely thymectomized five dogs which were six days to four months of age at the time of the operation. Three dogs died within two weeks after the operation. From his observations on the remaining two dogs, Friedelbein concluded that thymus was not essential to life, that it was concerned with blood formation and that thymectomy caused animals to grow faster. Although Friedelbein's work was imperfect, it had great influence on the field of thymus investigation. His experiments not only demonstrated that thymectomy was possible but also suggested the lines along which subsequent investigations have moved.

For the next thirty five years all efforts to discover the function of the thymus seem to have been dropped. Then Langerhans and Saveliew (1893) operated on 29 rabbits aged three to five weeks. They followed Friedelbein's method and removed the thymus by traction through an incision at the base of the neck. There is some doubt as to the completeness of the extirpations and so their conclusion that the thymus is not essential to life is not acceptable. Their one valuable contribution to the subject was the method. They were the first to control the operative animals with

others from the same litter.

Tarulli and Lo Monaco (1894) thymectomized several puppies of unknown age. The animals operated on were controlled by others from the same litter. It was noted that there was an increase in the number of eosinophils in the blood immediately following the operation. Several days later there was diminution in the hemoglobin and in the number of erythrocytes. These effects had entirely disappeared four months after the operation. In spite of abnormal appetite, the thymectomized dogs gained weight less rapidly than the controls, were much weaker and their hair lacked normal luster and strength. The retardation in growth was overcome at the end of six weeks and there was no noticeable difference between the operative and control animals at the end of three months. It is unfortunate that Tarulli and Lo Monaco did not give more detailed information in their written account of the experiment. Their omission of such important details as number and age of the animals operated on, weight increases and diet leads us to disregard their work.

Abelous and Billard (1896) selected the hitherto untried frog for their work because it was anatomically well adapted for thymectomy. Another reason for their selection was that the frog thymus persisted throughout life and might be expected to exercise an undiminished function during that entire period. In no case did the frogs from which both lobes of the thymus were extirpated live any longer than from three to fourteen

days after the operation. From 24 to 48 hours after the operation the animals showed lassitude, muscular weakness, progressive anemia and finally died. If only one lobe of the thymus was removed the animals showed no symptoms, except those due to surgical shock, and recovered fully. If the remaining lobe was removed, the animal died. When one lobe was removed, the other showed definite hypertrophy in twenty days. The authors claimed that injection of thymus extract, obtained from calves, caused completely thymectomized frogs to pass from a state of muscular weakness to one of hyperexcitability. As a result of their investigations they concluded that the thymus was essential to life. These results were confirmed by Camia (1900). Vincent (1903) refuted Abelous and Billard and Camia. Vincents' completely thymectomized frogs lived for at least thirty six days after the operation and then died of natural causes or were killed.

Ver Eeke (1899) found that the symptoms noted by Abelous and Billard did not occur if the water in the tanks was changed frequently. The completely thymectomized frogs recovered when moved to fresh and frequently changed water provided the symptoms had not progressed too far. Ver Eeke said that Abelous and Billards' frogs died because of contaminated water. He admitted that the fact that Abelous and Billard had used summer frogs whereas he had used fasting winter frogs might change the results.

Hammar (1905) found that removal of one or both lobes of the

thymus produced no effect in either summer or winter frogs. Hammar concluded that the thymus was not essential to life in the frog. Pari (1906) completed the evidence against Abelous and Billard's work by isolating an organism which had developed in the blood and organs of thymectomized frogs confined in unchanged water. Pari succeeded in producing the symptoms described by Abelous and Billard by inoculating both normal and thymectomized frogs with this organism. Pari concluded that a relationship existed between thymus function and immunity. Adler (1914) extirpated the thymus by means of the galvano-cautery in nine hundred and fifty tadpoles. At the end of three months there were twenty survivors. Examination disclosed that of these twenty only three were completely thymectomized. The testicles of these three were larger than the others and the thyroid exhibited hyperplasia and diminished colloid. There were no other abnormalities noted. Adler's lack of sufficient evidence did not deter him from stating, " A specific relationship exists in the frog between thymus and thyroid and between the thymus and the testes".

Carbone (1897) removed the thymus from several rabbits and one dog of unknown age. The animals exhibited no abnormalities except a slight diminution in the number of erythrocytes. Some doubt as to the validity of Carbone's work is aroused by Matti (1912) who says that the animals were too old to react to thymus extirpation. Cozzolino (1904) thymectomized twenty four rabbits weighing 172-665 grams at the time of operation.

Nine of the animals died of pneumonia within two weeks after the operation. The remaining fifteen showed no changes. Cozzolino's work has been cited by several writers as proof that thymectomy has no effect on growth and development. In reality his work is without any great significance. His first mistake was that he kept his rabbits for a comparatively short period after operation, about a month. Moreover, he deprived six of the rabbits of a value they might have possessed for the study of thymus function by injecting them with diphtheria toxin in an effort to gage the resistance of thymectomized animals to an acute disease. If confirmation of the fact that diphtheria toxin is fatal to rabbits was needed, Cozzolino's experiment gave this confirmation, but little else. As a result of his ambitious investigations, Cozzolino concluded that the thymus is not essential in postfetal life and that the thymus is not concerned in any way in the development of rickets.

Vincent (1903) thymectomized a series of guinea pigs aged ten days to one month. He found no effects whatsoever. Vincent was confirmed by Paton and Goodall (1904) who also worked on the guinea pig. Paton (1905) removed the thymus from twenty four guinea pigs aged one day to six weeks. He confirmed his previous experiment and also drew the specific conclusion that the thymus inhibits the growth of the testes in immature but not mature animals. The work of Fischl (1907) on the goat, rabbit, dog and fowl led him to conclude, as did many of his predecessors, that the thymus was without significance

to the organism. Nevertheless he did not give up the idea that the thymus was an organ of internal secretion but declared that it had a function in fetal life which ceased at birth. Bracci (1905) removed the thymus from eleven young rabbits with the object of determining the effect on calcium metabolism. Ten of the rabbits survived and were still alive four months after the operation. Four normal rabbits were used as controls. Half of the thymectomized animals received injections of thymus extract at irregular intervals. Bracci's estimations of calcium output and content of the organs in normal, thymectomized and thymus injected animals indicate a relationship between thymus function and calcium metabolism. The thymectomized animals showed a calcium excretion in excess of that which occurred before the thymus was removed and both thymectomized and normal animals showed a lowered calcium output immediately after receiving injections of thymus extract. The calcium content of bone, brain and blood was found to be less in thymectomized and greater in injected and normal animals. There were no changes in the other organs.

A distinct advance in the study of thymus function was made by Basch (1906) who greatly simplified the method of thymus removal. He selected the dog as the subject best adapted for his purpose and found that small dogs, such as the rat terrier and fox terrier, were the most suitable. He controlled from the same litters. Basch selected the third or fourth week of life as the best time for operation because he considered the

thymus to be at the height of its function during this period. He killed most of the animals in the second month after extirpation, but kept several pairs under observation for almost six months. His operative method consisted merely of an incision from the base of the neck along the middle of the sternum as far as its lower third, thereby exposing the thymus to view. Basch states that he lost no animal from the last five litters operated on. His records show that the animals from fourteen of the seventeen litters operated on suffered from softening of the long bones. The other three litters showed no changes. Basch concluded that the thymus is not essential to life but exercises a passing function. He considered it to be concerned chiefly with bone growth, development and calcification. To those accepting Basch's work at its face value, his experiments are enormously important in the history of internal secretion. They bring the thymus to a position among the most important and best understood organs of internal secretion.

However, since Basch's work assumes such great importance, it seems advisable to examine his results more closely. Basch advanced the theory that the bones of thymectomized animals lose some of their osteogenetic energy. To prove this, he broke the legs of both control and thymectomized animals and took roentgenograms at frequent intervals during the healing process. Basch felt that his theory was proven by the fact that the callus formations about the fractures in thymectomized

animals were shown to be smaller than those in normal animals. Closer examination of these roentgenograms reveals that the fractures are more severe in the control dogs than in the thymectomized, i.e, the line of fracture is the more distinct, the wider, or the displacement is greater. More than anything else, Basch's roentgenograms seem to indicate that callus formation is greatest where injury is greatest.

Careful reading of Basch's chemical and pathological descriptions gives the impression that some of the changes supposedly undergone by his thymectomized dogs were not very definite. For example, Basch states that his thymectomized animals exhibited a "straddling gait". This does not seem unnatural in an animal whose sternum has recently been split and whose pectoral and cervical muscles have been divided. Basch interprets the fact, that a dog whose thymus had been removed used his fractured leg two or three days before the control, as meaning that the thymectomized dog was pathologic. However, it seems entirely possible that this might indicate that the control was pathologic.

The closer the examination of Basch's findings, the more obvious it becomes that most of his impressions were subjective. If we eliminate these, then the only conclusion we can draw from his experiment is that thymectomy in the young causes a slight retardation in development. Park and McClure (1919) call attention to the fact that the pathologic condition in the bones of Basch's thymectomized animals was limited to the

legs. They consider that he was describing the effects of confinement and not thymectomy. These men say that if Basch was dealing with some general disturbance in bone metabolism dependent on thymectomy, the rapidly growing middle tier of ribs would have been affected with especial severity instead of remaining unaffected.

It is not the purpose of this writer to refute Basch's work. If his conclusions are correct, he has done a great service in bringing them forward.

Soli(1906) published a preliminary report of thymus extirpations in cocks and rabbits. Later, (Soli 1909) he presented more important papers dealing with the effects of thymectomy on the growth and development of the skeleton and of the testes in cocks, rabbits and guinea pigs. He removed the thymus from two rabbits, the first was one month old, the second was two months old. The thymectomized animals were controlled from the same litter. One of the animals was killed after an observation period of six weeks, the other after a period of three months. Soli states that the volume and length of the bones were less in the thymectomized animals than in the controls and concludes that the thymus exerts a regulatory influence over growth of bone in the young but not the adult animal. It is impossible however to accept Soli's results based as they are on observations made on two rabbits.

We now come to a consideration of the work of Klose and Vogt(1910) whose extirpations of the thymus, performed on no

less than five distinct species, had great influence in altering the course of scientific opinion in regard to the function of the thymus. They used ten litters of mongrel puppies totalling sixty four animals. Using Basch's method they thymectomized fifty four of the puppies, retaining one from each litter as a control. According to Klose and Vogt, the dogs exhibited a transient hyperplasia of the testes. Shortly after, the dogs began to lose weight, become dull and listless, exhibit an abnormal appetite and finally die in coma. Klose and Vogt put forward a theory of thymus function to explain the softening of the long bones so often demonstrated by thymectomized animals. They believed that the thymus is the chief organ of nuclein synthesis in fetal and early post-fetal life. When the thymus is extirpated, phosphoric or nucleic acid is no longer neutralized or combined. The accumulation of this acid gives rise to phosphoric acid acidosis which causes decalcification of the bones. Klose and Vogt's work is subject to the same criticizms as that of many of their predecessors. In the first place, there is doubt as to the completeness of the extirpations. Secondly, a general vagueness accompanies their work in that all those details, which science demands as a preliminary to acceptance of results or conclusions, are lacking.

Pappenheimer (1914a) with the aid of an apparatus to maintain intrapulmonary pressure, removed the thymus from rats aged three weeks or less. The control animals were from

the same litters. There were twenty six litters yielding a total of one hundred and eighteen animals. An epidemic of dysentery left him with eleven thymectomized and ten control animals. The period of observation after operation ranged from thirty one to one hundred and thirty one days. Every one of the test animals was proven to be thymus free by close examination of serial sections of the neck and upper half of the thorax. Pappenheimer found no disturbance of growth and development and no pathologic changes anywhere in the organs of internal secretion. Since no thymus rests were present, his negative results assume far greater importance than the positive results obtained by other men, for their results were possibly due to other causes than thymectomy. Pappenheimer (1914b) reports further results of thymectomy in rats. He repeated his previous experiments on a larger series of animals and again obtained negative results. He concluded that the thymus was not essential to life.

Hewer (1914) and (1916) was one of the first to experiment with the results of thymus feeding on white rats. She divided her thirty one rats into three groups. The first group was used as a control, the second was fed thymus tablets and the third was fed fresh lamb's thymus. They were fed their daily ration of thymus first and then given "ordinary food". The animals were killed and examined during a period of forty one weeks. The animals were found to be normal in all respects in all cases. Hewer concluded that there were no constant

changes due to thymus feeding.

Gudersnatch (1914) reported a series of experiments in which he fed a large number of tadpoles with fresh calves thymus. He states that this diet produced large tadpoles which metamorphosed late or not at all. This delay in metamorphosis was confirmed by Uhlenhuth (1918). However, he states that this delay may be overcome by supplementary feeding of plant food. Uhlenhuth's work seems to indicate that Gudersnatch was dealing with the effects of undernourishment brought on by an exclusive diet of thymus rather than any direct effect of the constituents of the gland.

Returning to the field of thymectomy, the investigations of Park and McClure (1919) take a prominent position. They thymectomized seventy five puppies between the ages of 9 days and 7 weeks. Each animal had its own control in 11 out of the 14 litters operated upon. After operation, the dogs were boarded with private families and observed at infrequent intervals. They found no alteration in the hair, teeth, contour of the body, muscular development, strength, activity or intelligence of the animals. Park and McClure concluded that the thymus gland was not essential to life. Although they feel quite sure that extirpation of the thymus does not influence growth or development or produce alterations in the organs of internal secretion, they do not entirely exclude this possibility. Their work has convinced many experimenters that the bone changes found by some workers after thymectomy were

due to rickets and not loss of the gland.

Downes and Eddy (1920) conducted a series of experiments on young rabbits in an attempt to discover the effects of subcutaneous injections of thymus substance. They used powdered dessicated thymus gland, one gram of which is said to represent approximately five grams of fresh calf thymus. The final preparation contained twenty milligrams of thymus substance per cubic centimeter of glycerine. The controls were injected with pure glycerine. Injections were given twice a week and the initial dose of two milligrams per one hundred grams of body weight was increased to a maximum dose of ten milligrams per one hundred grams of body weight at an increase of one milligram per week. For the first ten weeks the thymus treated animals gained weight faster than the controls, but during the second ten weeks the controls outstripped the thymus injected animals. Necropsy showed that the injections had caused an increase in the weight of the thyroid and spleen and a decrease in the weight of the thymus. Downes and Eddy concluded that subcutaneous injections of large doses of dessicated thymus substance, while checking the putting on of weight in young rabbits, did not otherwise impair growth or development. They offer no explanation for the changes in the weights of the thymus, spleen and thyroid.

Recent Investigations

Any division of the literature on thymus into past and present work must of a necessity be arbitrary. In this review, all work done during the last decade will be considered of

recent origin.

Chouke, Whitehead and Parker (1932) dissected seven human cadavers in an unsuccessful attempt to find a closed lymphatic system joining the thyroid and the thymus glands. Dissection of six dogs after injections of India ink into the thyroid also failed to show any connection between the two. Finally, they made sections of two puppies after injection of Gerota's fluid into the thyroid. The thyroid was massaged for fifteen to twenty minutes after injection in order to force the ink through any possible existing passageway. This experiment was as unsuccessful as the previous attempts. The authors concluded that any interaction between the thymus and thyroid glands must take place through the circulatory system.

One of the first attempts to discover the effects of thymus injection and implantation in rats was made by Ascher (1930). He used standardized white rats as his test animals. It was found that rats that were losing weight on a low vitamin diet returned to normal weight after injections of thymus extract. Ascher's extract was merely a water solution of thymus called "thymocrescin". If normal young rats were injected with thymocrescin their growth was extraordinary. The same results were obtained by implanting pieces of fresh thymus and administration of a growth producing diet. Two groups of rats were fed a rickets producing diet and one of the groups was given daily injections of thymocrescin. The controls contracted a bad case of rickets but the injected group was normal.

Ascher concluded that thymocrescin decreases calcium elimination in growing young rats. This decrease is a phenomenon associated with normal vertebrate growth.

Cataliotti (1931) totally extirpated the thymus in eight puppies six weeks to three months old. Within two months a depressed alkaline reserve made itself manifest and finally resulted in a distinct acidosis. Cataliotti considered the skeletal decalcification noticed by some workers as the result of an attempt to compensate for acidity of the blood plasma.

Gudersnatch (1930) reentered the field of thymus investigation and reported the results of feeding dried thymus gland to successive generations of white rats. He began the treatment as soon as the nursing period had passed. He reported that this diet had a highly beneficial effect on all test animals without exception. First, the rats were "very healthy," second, they were longer lived than the controls, third, pregnancies were more numerous, and last, litters were larger than in the case of the controls. The possibility that the thymus gland fed to the rats acted merely as any good growth producing food might have acted leads most investigators to accept his work with reservations.

A very complete report of the results of thymectomy in white rats was published by Andersen (1931). She thymectomized 86 female rats and 40 male rats controlling from the same litters. The diet and living conditions were standardized.

The opening of the vagina was used as the criterion of puberty in the females and the presence of motile sperm in the tail of the epididymis was the criterion of puberty in the males. The experiment was divided into three parts so as to control for seasonal differences. Series 1, consisted of 26 operated and 26 control rats which were born in the winter and operated on during the third of fourth week of life. Series 2, consisted of 39 operated and 40 control rats born in the spring and thymectomized on the twenty first day of life. Series 3, consisted of 61 operated and 61 control rats born during the winter and spring and operated on during the first day of life. All three series, both operated and control animals included, reached puberty within a few days of each other. The weight differences between operated and control animals were just as negligible, usually varying no more than ten grams either way. These experiments which were controlled as to diet, living, conditions, season and completeness of thymectomy, demonstrate that deprivation of thymus function does not affect the age of puberty in either male or female rats.

The effect of feeding dessicated thymus on growth was investigated by McKay and Barnes (1934). They wished to discover whether the results reported by Gudersnatch (1930) were obtainable only by administration of thymus or whether they could be obtained by administration of some other tissue. The addition of a significant amount of dessicated beef liver or kidney to the diet of albino white rats impaired the rate of growth slightly. Dessicated beef thymus was found to be even

more deleterious in effect. McKay and Barnes thought these effects were due to the nuclear material in the tissues because their harmful results seemed to be in direct proportion to their content of purine nitrogen. They concluded that Gudersnatch's results must have been due to other causes than administration of thymus gland.

Any consideration of recent investigation concerning the function of the thymus gland must, of a necessity, include the work of Rowntree and his colleagues. A close examination of their publications reveals that their experiments were divided into five parts. Each of these parts will be taken up in a different section.

Throughout the whole series of experiments, standardized white rats of the Wistar strain were used. The animals operated upon were controlled from the same litters. Care was taken to standardize the diet and living conditions. Each experiment has had the same basic principle, that is, continuation of each adopted procedure through several succeeding generations of rats. In the first part of their experiment Rowntree (1934) injected an extract of thymus prepared from fresh material obtained from calves. The extract was a suspension of finely ground fresh thymus suspended in a physiological salt solution (Hanson, 1934). The test animals were subjected to one cubic centimeter of the extract injected intraperitoneally, daily, even during pregnancy and lactation. During the past five years, 1240

litters totalling more than 8000 rats, have been treated in this manner. The authors claim that the injections increase the weight of the prepubertal male and mated mature female but exert no noticeable effect on the new born. Treatment of parent rats seems to increase the number and size of the litters and the weight of the young at birth. As a result of carrying the above mentioned procedure through twelve successive generations of rats, Rowntree concludes that injection of thymus extract results in accruing acceleration in the rate of growth and development in succeeding generations and in increased fertility of parent rats. It was found that interruption of thymus administration for one generation nullified, to a great extent, the effects of previous thymus treatment even though the treatment had extended through several generations of rats. The appended chart shows more clearly than words how startling were the results of thymus injection through eleven successive generations. Rowntree (1936)[a] found that extracts over a month old were often inert. No definite reason has been offered in explanation of this inertness.

The second part of their experiment was devoted to an attempt to remove the thymus gland. Four pairs of rats were anesthetized and the gland was removed intact. The operation was performed when the rats were 17-24 days of age. All surviving animals were weaned at 21-28 days of age. In order to control the results in offspring of thymectomized rats, a study was made of a special group whose parents were subjected to the

surgical technicque concerned in thymectomy but in whom the thymus was not actually removed. This group of rats showed no significant deviation from normal controls. The original four pairs of thymectomized rats bred for five generations and yielded 555 young which were also thymectomized. Rowntree (1935)and (1936) states that his test animals showed a definite retardation in growth and a slight retardation in the rate of development which proceeded with accruing acceleration. The retardation was greatest between the ages of 10 and 50 days. At.70 days the average weight of the thymectomy test strain in all five generations was still below the average weight of the controls, but at 120 days, the weights of the test and control groups were practically identical. The appended chart is supposed to show wherein lies the retardation noted.

In the third part of his experiment, Rowntree (1934) made implants of fresh thymus at 5-7 day intervals in young rats of each of five successive generations, beginning at 25 days of age. As the before mentioned chart will show, an accruing acceleration in growth and development has been observed in this group, comparable to that noted in the thymus injected strain.

The fourth experiment concerned itself with the effects of first removing the thymus gland and then replacing by means of injections of Hanson's thymus extract in daily intraperitoneal, doses of one cubic centimeter. The young of each generation were thy-

thymectomized at approximately 20 days of age and then mated and injection of extract begun. This procedure was carried on for five successive generations. It was found that the retardation caused by thymectomy was more than overcome by the amount of extract used, since each generation of rats showed a rate of growth and development in excess of normal. Cf. Chart.

The fifth and last experiment was conducted exactly as the fourth with the exception of the replacement therapy employed. In this case the thymus was replaced by means of implants taken from normal 20-40 day old rats. Rowntree (1936b) observed that the retardation caused by extirpation of the thymus was more than overcome by this method of replacement since each generation of rats showed a rate of growth and development in excess of normal. Cf. Chart.

Rowntree points out that there is an accruing nature in the effects of each procedure described. "Amplification becomes more pronounced with passage through several successive generations". The explanation for this amplification is not yet evident but it is known that treatment of both parents is necessary to maximal effect. Rowntree concludes that the thymus gland is concerned with the rate of growth and development in the young.

This review has attempted to describe briefly the work of the men who have had the most influence in directing scientific

opinion as to the function of the thymus. Unfortunately, recent investigations do not seem to have brought the problem any nearer solution than those of earlier times. The reasons for this confused state of knowledge are plain. The methods of investigation have been decidedly inferior, especially in the field of thymus removal.. The work of Park and McClure (1919) has shown that Friedelbein's method of thymus removal is seldom if ever entirely effective. Therefore, the experiments of earlier and more recent investigators who have used this method cannot be accepted as valid. Some investigators have used better methods but have failed to use suitable subjects or a sufficient number of control animals. Too many of the results reported rest on the authority of the investigator's word alone. Experiments so limited that normal variation and environment cannot be excluded as the reasons for change, have not deterred some workers from drawing hard and fast conclusions which have only added to the confusion. It is quite evident that some workers have begun their experiments with the assumption that the thymus is possessed of some function which can be shown by removal. Therefore they have over valued their positive results and practically ignored their negative results. In this way a great difference is found between the actual results of the experiments and the conclusions drawn from them.

On the basis of past work, it seems that negative results are far more important than positive because, as we have seen,

the results usually laid to thymectomy may be brought about in other ways.

It should not be assumed that the causes given above are the only reasons for the confused state of ideas concerning the function of the thymus. It is quite probable that most of the difficulty lies in the problem itself. However, in the face of such varied results from the same procedures, it seems quite evident that either the problem is difficult or the thymus is possessed of no function, or if the thymus has a function, then it certainly can not be demonstrated by present methods of investigation.

The amount of certain knowledge concerning the effects of thymus removal is surprisingly small. The work of Hammar (1905) has proven that it is not necessary to life in the frog and that its complete removal results in no detectable change in growth and development in that animal. Pappenheimer (1914) states that the extirpation of the thymus produces no changes in the rat. In view of the fact that he proved his rats to be thymus free by means of serial sections of the neck and thorax, his work on this phase of thymus investigation seems preferable to that of Rowntree.

The reported effects of extirpation of the thymus can be placed in any one of three groups, (1) thymectomy has no effect (2) thymectomy causes passing disturbances in growth and development (3) thymectomy causes death with or without changes in growth, development, skeleton and organs of internal se-

cretion. Quite a few observers have been unable to detect any change following thymectomy and no one has proven that thymectomy causes death.

The reports of observers on the effects of thymus administration can also be divided into three groups,(1) Thymus injection, implantation or feeding causes a marked increase in the rate of growth and development (2) Thymus administration in appreciable quantities causes death or retardation in the rate of growth and development (3) Thymus administration has no other effect than that of a good food. Almost all who have reported positive results have obtained negative results as well. In a discussion of the results of injecting thymus extract into ten successive generations of white rats, Rowntree (1935) says,"With original extracts, the effects upon acceleration of growth and development were extremely consistent. Since that supply was exhausted and we have been using extract made up in the Philadelphia Institute, there has been more variability especially in weight."

The reports of some investigators seem to indicate that thymus extirpation affects but a few organs and those of others show that it affects the whole body. The positive results of some contradict or show little similarity to the positive results of others. The positive results of a certain group have a similarity in that they center about changes in the bones. These changes are, retardation in the growth of the whole skeleton,retardation in growth and length of the long

bones, osteomalacia, osteoporosis,and rickets.

Hardley any have studied the organs ofinternal secretion with care following either extirpation or administration. Many present day investigators have not considered it worth while or necessary to apply some of the more recent histologic methods tothe study of the thymus gland. In view of the fact that the positive evidence for thymus function is far out-weighed by negative evidence, we are forced to the conclusion that the thymus is not necessary to life and that an essential relation to the normal processes of growth and development has not been demonstrated.

Summary

Beginning with Restelli in 1845, a series of attempts have been made to discover the function of the thymus. The enormous mass of literature describing the effects of thymus administration and removal has brought the problem no nearer solution. The positive findings attributed to thymectomy could be due to chance variation, unfavorable living conditions, improper diet or disease. Too many workers seem to have proceeded from the assumption that all changes in their animals were due to thymectomy and that the operation was a negligible factor. However, it is evident that an animal confined in a cage or cellar while still weakened from operation might naturally show retarded growth and development. There is nothing striking in such a disturbance of growth and development as the onset of rickets. It is a well known fact that confinement without sunlight causes rickets. As a matter of fact, the symptoms and changes usually attributed to thymectomy are practically the same as these which occur in animals as a result of confinement, improper diet and parasitic or bacterial infections.

Turning to a consideration of the results of thymus administration, one is met by a series of results as widely divergent as those obtained in the field of thymus extirpation. The workers who report that thymus administration has no effects or produces effects similar only to those of a good food seem to have more solid basis for their contentions

than those who report startling acceleration in the rate of growth and development. The work of Rowntree dominates this field and it may be that he has solved the problem of thymus function. However, before we can accept his statement that injection of thymus extract results in accruing acceleration in the rate of growth and development, confirmation will be necessary. Therefore, we may conclude that the administration of thymus gland has no specific effect on the rate of growth and development other than that of a good food.

Somatic Development in Advanced Generations of Treated Animals

	Birth weight in grams	Ears opened in days	Incisors Erupted in days	Hair Appeared in days	Eyes opened in days	Testes descended in days	Estrus in days	Motile sperm first detected days	Vagina opened in days
Controls	4.9	2½-3½	9	14-16	14-16	25-35	70-80	60-70	41-50
Results of thymus injection at F_{11}	6.0	Birth-1	Birth-1	1-2	1-2	4-6	9-18	—	6-9
Results of Thymus Extirpation at F_5	4.6	3	13	14	16-19	30-33	62	—	44-62
Thymus Implants in Normal Rats at F_4	5.1	2-3	2-3	6-8	9	20	—	—	36
Thymus Implants from Normal Rats into Thymectomized at F_3	5.6	2	4	6	7-8	20	—	—	36
Thymus injections into thymectomized at F_4	5.0	3	6	8	12	24	—	—	40

Literature Cited

Abelous, J.E. and R. Billard. 1896. Recherches sur les fonctions du thymus chez la grenouille. Arch. de physiol. norm. et path. 8: 898.

Adler,L. 1914. Extirpation of the Thymus. Arch. f. Entwcklgsmechn. d. Organ. 40: 1.

Allen, B.M. 1920. The results of earliest removal of the thymus glands in Rana pipiens tadpoles. Journ. Exp. Zool., 30: 189.

Andersen, D.H. 1931. Studies on the physiology of reproduction. Journ. Physiol. 43: 184.

1932. Relationship between Thymus and Reproduction. Physiol. Reviews, 12: 1.

Badertscher, A. 1914. Development of the Thymus in the Pig. Am. J. Anat., 17: 437.

Basch, K. 1908. Beiträge zur Physiologie und Pathologie der Thymus. Jahrb. f. Kinderh, 68: 668.

Boyd, E. 1936. Involution of the Thymus. Am. J. Dis. Child. 51: 313.

Bracci, C. 1905. Timo e ricambio de calcio. Riv. di clin. pediat. 3: 572

Camia, M. 1900. Richerche sulla funzione del timo nelle rane. Riv. di patol. nerv. 5: 97.

Calzoliri, A. 1898. Richerche sulla funzione del timo.Arch. ital. de biol., 30: 71

Carbone, T. 1897. Sull' estirpazione della ghiandolo timo.

Gior. d. r. Acad. di med. di Torino. 45: 561.

Cataliolti, R. 1931. Effect of Thymectomy on the Alkaline Reserve. Bio. Abst. 7: 1, No. 13073.

Chouke, K. Whitehead, R. and A. E. Parker. 1932 Is there a closed lymphatic system joining the Thyroid and Thymus? Surg. Gynec. and Obstetr. 54: 6, 865.

Cozzolino, O. 1904. Risultati della stimolazione nei giovani conigli. Lavori d. Cong. di med. int. Padova 13: 230.

Downes, A. W. and N. Eddy. 1920. The effects of subcutaneous injections of thymus substance in young rabbits. Endocrinology 4: 420.

Einhorn, N. H. and L. G. Rowntree. 1936. The results of thymectomy in successive generations of white rats. Science 83: 443 May 8.

Fischl, R. 1907. Extirpation of the Thymus in the young. Monatschr. f. Kinderh. 5: 329.

Friedelbein, A. 1858. Der Physiologie der Thymusdrüse. Frank. a. M. lit. Anstalt. 366 pp 8.

Gudersnatch, F. 1914. Feeding experiments on tadpoles. Am. J. Anat. 15: 431

1930. Feeding rats on glands of internal secretion. Anat. Record, 14: 35.

Hanson, A. H. 1936. The Biologic Effects of Active Thymus Extract. Minn. Med. 19: 1.

Hammar, J. A. 1905 Die Thymusdrüse beim Frosch. Arch. f. d. ges. Physiol. 110: 337

1905a Zur Histogenese der Thymusdrüse. Anat. Anz. 27: 23

Job, T.T. 1922. Studies on lymph nodes. Am. J. Anat. 31: 125.

Klose, H. and H. Vogt. 1910. Klinik und Biologie der Thymus. Beitr. z. klin. chir. 39: 1

Matti, H. 1912. Physiologie und Pathologie der Thymusdrüse. Ergebn. d. inn. Med. u. Kinderh. 9: 1.

Pappenheimer, A. M. 1914a, The effects of early extirpation of the thymus in rats. J. Exp. Med. 19: 319.

1914b. Recent Advances in Thymus Research. Am. Med. 9: 212.

McKay, E. and R. H. Barnes. 1934. The effect of feeding dessicated thymus on growth. Soc. Exp. Biol. and Med. 32.

Pari, G. A. 1906. Sulla diminuzione dopo l'estirpazione dei timi. Transl. Arch. ital. de biol. 46: 225.

Park, E. and R. D. McClure. 1919. The results of thymus extirpation in the dog. Am. Journ. Dis. Child. 18: 317.

Paton, D. N. 1905. The Relationship of the Thymus to the Sexual Organs. J. Physiol. 32: 28.

Paton, D. N. and A. Goodall. 1904. Contributions to the Physiology of the Thymus. J. Physiol. 31; 49.

Restelli, D.A. 1845. De thymo observationes. Transl. Park and McClure, Am. J. Dis. Child. 1919, 18, 317.

Rowntree, L. G. Clark, J. H. and A. H. Hanson 1934a Biologic Effects of Thymus Extract Am. J. Physiol. 109: 94.

1934b. Science. 80, 274.

1934c. Tr. Assoc. Am. Phys. 49: 252.

1934d Journ. Am. Med. Assoc. 103, 1425.

1935. Accruing Acceleration in Growth and Development in successive generations of white rats. Arch. Int. Med. 56:1

1936a. Further studies on Thymus and Pineal Glands. Penn. Med. J. 606, May 1936

1936b Further Studies on the Thymus Gland. Ann. Int. Med. 9: 359.

Scott, G.G. and J. I. Kendall. 1935. The Microscopic Anatomy of the Vertebrates. Lea and Febiger, Phila.

Soli, U. 1907. Comportamento dei testicoli negli animali. trans. Arch. ital. di biol. 47: 115.

1909. Contributo alla funzione del timo. transl. Arch. ital. di biol. 52: 353

Tarulli, L, and D. Lo Monaco, 1894. 2: 19. transl. Park and McClure, Am. J. Dis. Child. 1919, 18, 317.

Uhlenhuth, E. 1918. The influence of thymus feeding. Journ. Exp. Zool. 25: 135

Ver Eeke, A. 1899. Contribution a l'anatomo- physiologie du thymus chez la grenouille. Bull. Acad. roy. de. Belg. 13, 67.

Vincent, S. 1903. The Results of Extirpation of the Thymus. Proc. Physiol. Soc. Lond. 30: 16.